ORIGINAL ARTICLE

Clinical Spectrum and Sensitivity Pattern of Isolates in Pediatric Febrile Neutropenic Patients of Acute Lymphoblastic Leukemia

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ABSTRACT

Aim: To determine the clinical spectrumand sensitivity pattern of isolates in pediatric febrile neutropenic patients of Acute Lymphoblastic Leukemia.

Methods: This descriptive observational study was conducted at Department of Pediatric medicine, The Children's Hospital, Lahore. A total of 100 patients between 01 to 15 years of age who fulfilled the criteria of febrile neutropenia were included in the study. Data was entered andanalyzed by SPSS 20.

Results: Out of 100 patients, 65(65%) were males while 35 (35%) were females. Forty fivewere between 1-5 years of age, 31between 6-10 years and 24were between 11-15 years of age. Majority (46%)presented with fever of unknown origin.Out of 160 cultures, 60 turned out to be positive. Highest yield was obtained from blood (45%). Forty eight were gram-negative (80%) while 9 were gram-positive bacteria (15%). Gram-negative bacteria were dominated by Klebsiella while Staphylococcus Aureus was the most frequently isolated gram-positive organism The sensitivity pattern of Klebsiella against empirical antibiotic regimen revealed highest sensitivity with Meropenem (31%). Highest sensitivity of Pseudomonas was found for Tazobactum/Piperacillin (77%). Staphylococcus Aureus was found to be 100% sensitive to Linezolid and Vancomycin followed by Amikacin .

Conclusion: Fever of unknown origin was the most common presentation. Gram negative bacteria dominated the isolates. The current empiric antibiotic regime has shown promising results in sensitivity patterns and should be continued till further studies.

Keywords: Febrile Neutropenia, Acute Lymphoblastic Leukemia, Antibiotics, Sensitivity

INTRODUCTION

Advances in chemotherapy of Pediatric acute lymphoblastic leukemia (ALL) patients using multi-agent chemotherapy has improved overall survival. Over the year's infections have emerged as a major cause of morbidity and mortality as patients on chemotherapy are at high risk of developing febrile neutropenia. During the last decade the survival of patients with fever and neutropenia has continued to improve due to availability of variety of antimicrobials agents[1]. Fever is the sole manifestation of infection in such patients, without any obvious clinical focus or microbiological cause and any delay in antibiotic therapy allows for unchecked progression of infection so adequate patient evaluation and sampling have diagnostic, prognostic and treatment related consequence[2]. Microbiological agents causing infections in these neutropenic patients include not only bacteria but also fungi and viruses.

Thestandard of care in febrile neutropenic patients is that they should be hospitalized if high risk and should be treated urgently with intravenous wide spectrum antibiotics. Empiric treatment should be modified according to culture results and clinical situation. Options for low risk patients are to start intravenous treatment and continuing with per oral treatment or to give per oral antibiotic from the beginning[3,4].

Use of broad-spectrum antibiotics increases cost of treatment, therefore every centre should find out a cost effective programme based upon most prevalent microbial flora in that centre. Because results of culture and sensitivity are available after a few days, it is imperative that institutions be aware of predominant flora in their set up, so as to formulate an effective empirical antibiotic regimen[5,6].

Grams-negative infections were thought to be the most common offending agents and usually a combination of aminoglycosides and beta-lactam antibiotics resulted in defervesence. However in the past few years, with increasing usage of indwelling catheters, use of intensive and prolonged chemotherapy regimens and effective treatment of gram negative infections, there has been a shift in the spectrum of bacterial isolates. Gram-positive organisms are now isolated with increasing frequency and Gram-negative organisms display more resistance to beta lactam and other antibiotics[7,8]. Shift towards Gram-positive organisms and continuing need to provide Gram-negative cover which are still common in developing countries and associated with high mortality demand the use of agents which provide coverage for the spectrum of potential infecting organisms[9].

The purpose of this study was thus to identify the major bacterial pathogens that are responsible for infections in neutropenic patients of ALL in our hospital and to look at their sensitivity patterns to empirical antibiotics employed here.

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PATIENTS AND METHODS

This descriptive cross sectional study was conducted on patients of ALL between the ages of 01 to 15 years who developed febrile neutropenia and admitted in pediatricmedicalbetween August 2019 to December 2019. Patients of ALL who presented with relapse were excluded from study. Febrile neutropenia was defined as fever of 38.3°C as a single reading or two readings of 38°C taken 2-4 hours apart and absolute neutrophil count of <500 cells/µL or < 1000 cells/µL but expected to fall. Diagnosis of ALL was based on clinical examination, peripheral blood picture, and bone marrow aspiration or trephine bone biopsy along with morphological French American British (FAB) classification. History was taken and thorough physical examination was carried out. Blood samples were drawn for complete blood counts. Cultures were taken from sites at risk and included blood , urine , skin swab, ear swab and sputum depending upon systemic involvements. All this information was gathered through a proforma and entered. At the same time empirical antibiotic regimen was started on all patients and subsequent course of these antibiotics was decided according to culture and sensitivity results and clinical response of patient; otherwise continued as such. The sensitivity of empirical antibiotics therapy was evaluated against the culture-isolated G ram-positive and Gram-negative bacteria. The empirical antibiotics used in our setup were Tazobactum-Piperacillin, Amikacin (first line), Salbactum-Cefoperazone and Ciprofloxacin (second line), Meropenem (third line) as well as Co Amoxiclav, Vancomycin, Linezolid and Third Generation Cephalosporins.

Data was analyzed using SPSS version 20 and statistical analysis was done.

RESULTS

Hundred patients of ALL were entered in study while they were febrile and neutropenic. In our study 65 patients (65%) were males while 35 patients (35%) were females. Forty five patients were between 1-5 years of age, 31 patientsbetween 6-10 years and 24 patients were between 11-15 years of age. Forty-six patients (46%) presented with fever only without any obvious focus of infection, followed by 20 patients (20%) with skin involvement in form of rash, cellulitis or ulcers. Rest of the data analysis revealed that diarrhea and pneumonia were the presenting feature in 12 patients each (12%) and acute otitis media in 10 patients (10%). (Figure 1). Out of 160 cultures obtained from 100 patients, 60 cultures turned out to be positive. Highest yield was obtained from blood (45%) followed by skin swabs (40%), urine (28%) and ear swabs (17%) None of the sputum cultures were positive. (Figure 2).Among the isolated bacteria, 48 were gram-negative(80%) while 9 were gram-positive bacteria (15%).Gram-negative bacteria were dominated by Klebsiella (26%) followed by Pseudomonas (15%), E coli (13%) and Acinetobacter (8%).Staphylococcus Aureus was the most frequently isolated Gram-positive organism (15%) followed by Streptococcal species (3%).(Table 1).

The sensitivity patternof Klebsiella (n=16) against empirical antibiotic regimen revealed highest sensitivity with Meropenem (31%) followed by Amikacin (18.75%), ciprofloxacin and Tazobactum-Piperacillin (12.5% each) and lowest sensitivity with third generation cephalosporins (6.25%). Highest sensitivity of Pseudomonas(n=9) was found for Tazobactum-Piperacillin (77%) followed by Salbactum-Cefoperazone and Ciprofloxacin(66% each), Meropenem (55%) and 44% for Amikacin and Third Generation Cephalosporins.

Staphylococcus Aureus was found to be 100% sensitive to Linezolid and Vancomycin followed by Amikacin (77%), ciprofloxacin, third generation cephalosporin and Co Amoxiclav (66% each), fair sensitivity to Third Generation Cephalosporins (55%) and 44% for Benzyl Penicillin.(Table 2)

Figure 1: Clinical presentation of Febrile Neutropenic patients (n=100)

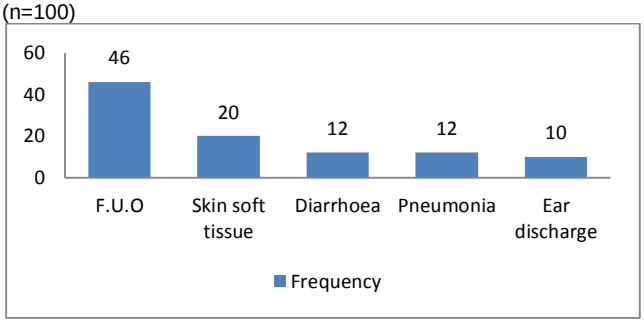

Figure 2: Distribution of Cultures and their Yield No. of Cultures obtained = 160, No. of Positive isolates = 60

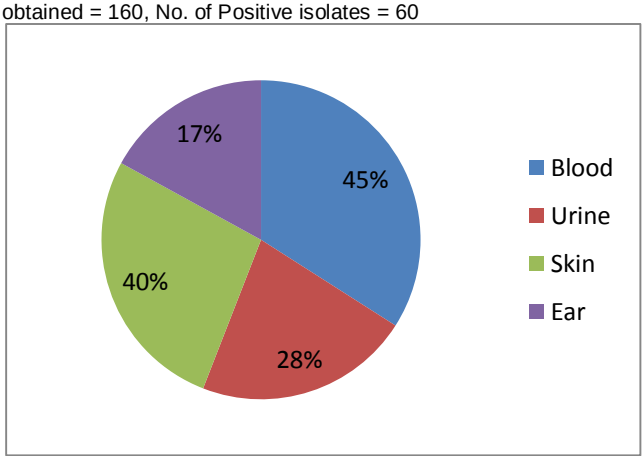

Table 1: Distribution of Bacterial Isolates (Total number of patients = 100) Number of Positive Isolates = 60

Isolated Bacteria	Number	Percentage
Gram-negative Isolates	48	80
Klebsiella	16	26.6
Pseudomonas	09	15
E coli	08	13.3
Acinetobacter	05	8.3
Citrobacter	03	05
Enterobacter	03	05
Salmonella	02	03
Proteus	01	1.6
StenotrophomonasMaltophilia	01	1.6
Gram positive isolates	12	20
Staphylococcus Aureus	09	15
Streptocouss	02	03
Enterococcus	01	1.6

Table 2: Sensitivity of bacterial isolates to commonly used antibiotics Total positive isolates= 60

Antibiotics	Klebsiella		Pseudomonas		Staph Aureus		E coli		Acinetobacter	
	N=16		N=9		N=9		N=8		N=8	
	N	%	N	%	N	%	N	%	N	%
Penicillin					4	44				
Co-Amoxiclav					6	66				
Tazobactum/Piperacillin	2	12	7	77			4	50	3	60
Salbactum/Cefoperazone	1	6	6	66			5	62	4	80
Linezolid					9	100				
Vancomycin					9	100				
Amikacin	3	18	4	44	7	77	8	100	3	60
Ciprofloxacin	2	12	6	66	6	66	2	25	4	80
Levofloxacin	2	12	7	77					2	40
Cefuroxime	1	6			6	66			1	20
Ceftriaxone	1	6			6	66			1	20
Ceftazidime	1	6	4	44	5	55	2	25	1	20
Meropenem	5	3	5	55			8	100	1	20

DISCUSSION

Infectious complications are an important cause of morbidity and mortality in pediatric patients of ALL presenting with febrile neutropenia.It is an oncological emergency, and initiation of antimicrobials at the earliest is necessary. The choice of empiric antimicrobial therapy and site of care (inpatient versus outpatient) is in part determined by the etiology of febrile neutropenia, time to establish the diagnosis and risk of related complications and death.

In our study, fever without any focus was the manifestation of infection in most of febrile neutropenic patients (46%). In a study from Turkey out of 200 episodes of febrile neutropenia, fever of unknown origin being the most common presentation, was observed in 46 episodes (23%).[10] Llamas RMH reports the same from Mexico (27.9%).[11] Granulocytes play an important role in the development of inflammatory responses to infection, and consequently during neutropenia, inflammation may be markedly attenuated despite ongoing infection. The presence of infection may be detected only by attention to seemingly minor complaints from the patients or by subtle localized physical findings.

The second most common presentation in our study was skin/soft tissue infections (20%). The frequent use of peripheral intravenous lines and their improper maintenance partly because of excessive work load in our set up was responsible for skin/soft tissue infections as skin and mucous membranes are most common portals of entry for microorganisms in these patients.Some studies have shownrespiratory tract infections to be the most common presentation of febrile neutropenic patients[4].

For microbiological diagnosis, cultures were taken from sites at risk. Samples included cultures of blood, urine, skin and ear swabs and sputum. In our study 40 microorganisms were isolated from blood cultures (45%), followed by skin swabs, urine and ear swabs. None of the sputum cultures turned out to be positive.Latest study done in India shows that prominent site of infection in neutropenic patients was bloodstream followed by urine and stool[12].

In our study Gram-negative bacteria were cultured more frequently than Gram-positive bacteria. Out of 60 isolates, 48 (80%) were Gram-negative and 15 (38%) were Gram-positive organisms.Latest Studies done from other centres in Pakistan like Shaukat Khanum Cancer Hospital[13] and National Institute of Blood Diseases[14] also showed the predominance of Gram negative isolates. SA et al[15] reported that out of 25% positive blood cultures the maximum isolation was of Gram negative bacteria. Same is the case from another developing country Bangladesh.[16] India[17,18] and Turkey,[10,19,20] have reported variability in the results of some centres where Gram-positive isolation is prominent. Overall a few centres are showing Gram-positive predominance like the study done in Thailand,[21] Mexico,[11] Saudi Arabia,[22] Iran[23] and USA.[24] Yao J e tal[25] from China has reported 60% isolated bacteria as Gram-negative isolates. This is evident from above findings that results vary between developing and developed countries, from centre to centre within the same country as well as increasing burden of Gram negative isolates in developing countries. The spectrum of organisms in febrile neutropenic patient is daunting as virtually any organism can cause invasion if host defenses are severely impaired.Both Gram-positive and Gram-negative bacteria are commonly isolated from blood sample of these patients. Over the past decade change in microbial spectrum from Gram-negative to Gram-positive bacteria is well documented. Western literature reports an increase in gram-positive infections in last decade or so although gram-negative infections still prevail. Factors that may be contributory are use of prolonged and intensive chemotherapeutic regimens, widespread use of prophylactic antibiotics against gram-negative organisms and hospital environment. Increasing frequency of skin/soft tissues infections due to chemotherapeutic drugs including Cytosine Arabinoside, deep and prolonged neutropenic attacks, prophylactic treatment with flouroquinolones and Co trimoxazole, use of antacids/histamine blockers as well as the use of intravenous lines is considered by other researchers to be responsible for increasing numbers of Gram-positive isolates. Nevertheless Gram- negative bacteria continue to be the main cause of bacteremia in developing countries and are associated with high mortality rate and coverage for Gram- negative isolates should be included in empirical antibiotic therapy.

Klebsiella was the most common Gram-negative bacteria isolated in our study followed by Pseudomonas, E coli and Acinetobacter. Staphylococcus Aureus was the

most commonly isolated Gram-positive microorganismfollowed by streptococci .Most of the latest studies from Pakistan are also supporting the predominance of Klebsiella in their cultures.[15,16,26] Similar results were shared in the region by India,[17,18] Iran,[23] Bangladesh[16] and Turkey.[10,19,20] Like our study all the three latest Turkish studies[10,19,20]and Iranian study[23] had Staphylococcus Aureus as their most common Gram-positive isolate.

Empirical antibiotics tested in our study included Piperacillin-Tazobactum and Amikacin as the first line antimicrobial agents followed by Salbactum-Cefoperazone and Ciprofloxacin (second line) as well as Meropenem (third line). Other antibiotics which were employed depending upon the site of infection and nature of bacterial isolate included Vancomycin, Linezolid, Co Amoxiclav and Third Generation Cephalosporins. Klebsiella (n = 16) was found to show best sensitivity to Meropenem (31%) followed by Amikacin (18%), Ciprofloxacin and Piperacillin/Tazobactum (12%). There was poor sensitivity to Third Generation Cephalosporins (6%).Same results have been shared by SA et al[15] from Pakistan where Klebsiella was found to be sensitive to all empirical antibiotics except third generation cephalosporins but Pseudomonas responded well to 3rd generation cephalosporins. Abraham NL[12] from India reported that species such as Ecoli, Klebsiella and Enterococcus were sensitive towards first-line agents such as Piperacillin-Tazobactum alone or in combination with Amikacin. Second-line agents such as Imipenem, Meropenem, or Linezolid alsohelped significantly to reduce the episodes of febrile neutropenia.In our study Pseudomonas was found to be sensitive to Piperacillin-Tazobactum and Ciprofloxacin (77%) followed by Salbactum-Cefoperazone and Ciprofloxacin (66% each), Meropenem (55%), Amikacin and Third Generation Cephalosporins (44%).Yeamin MB et al[16] from Bangladesh has reported the same sensitivity pattern. E-Coli was 100% sensitive to Amikacin and Meropenem followed by Salbactum-Cefoperazone and Piperacillin-Tazobactum. There was poor sensitivity to Third Generation Cephalosporins and Ciprofloxacin (25% each). Acinetobacter showed good sensitivity to Salbactum-Cefoperazone and Ciprofloxacin (80% each) followed by Piperacillin-Tazobactum and Amikacin (60% each). They did not respond well to Meropenem and Third Generation Cephalosporin (20% each).Recent study from Japan and Korea also showed overall good results with Piperacillin-Tazobactum and Meropenem for the eradication ofGram negative isolates.[27,28]

Staphylococcus Aureus showed 100% sensitivity to Vancomycin and Linezolid followed byAmikacin (77%), Co-Amoxiclav, Ciprofloxacin and Third Generation Cephalosporins (66% each). Similar results were shared by Bishar A et el.[29]

Thus frequent surveillance of bacterial pathogens responsible for microbiologically defined blood stream infections and their respective antibiotic susceptibilities is central to tailoring empirical antibiotic therapy in febrile neutropenic episodes.

Our study has certain limitations including lack of expertise and technical errors, which may be the factors for most of the cultures being negativeand there is no facility for detection of viruses in our set up.

CONCLUSION

This study concludes that fever without any focus is the major manifestation of ALL patients with febrile neutropenia. Gram-negative organisms dominated the cultures with Klebsiella being the most common isolate followed by Pseudomonas, E coli and Acinetobacter. Staphylococcus Aureus was the most common Gram-positive bacteria isolated. The current empiric antibiotic regimen consisting of Co- Amoxiclav, Tazobactum-Piperacillin and Amikacin, Salbactum-Cefoperazone and Ciprofloxacin and Meropenemhas shown promising results in sensitivity patterns and should be continued till further studies.

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